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Description

I. Field of the Invention

The invention relates to test compositions and test devices capable of generating different hues at different analyte concentrations. Visual tests for clinical analytes are the focus of the invention.

II. Utility

Colorimetric tests are conveniently used as visual tests with which relatively untrained personnel can routinely obtain results by simple comparison to an appropriate color chart. Visual tests are inexpensive and convenient since no instrumentation is required. Presently, visual tests are used for routine screening of urine samples for a number of diagnostically important analytes, used by diabetics for home testing of urine or blood glucose and used in other fields, for example water testing for iron content.

However, currently available visual tests relate the intensity of a particular color to the concentration of analyte. For example, a test device may change from colorless to light blue to darker shades of blue with increasing concentration of glucose. Greater visual discrimination, and therefore greater accuracy, is possible, when a range of colors is provided rather than different shades of a single color. Therefore a test composition which exhibits different colors (or hues) at different analyte concentrations would be easier to use and would provide more accurate visual results.

In order to allow visual differentiation of higher concentrations of glucose, many currently available products resort to the use of two reagents pads, one of which provides better color differentiation at the higher concentration range. Otherwise such products would exhibit only very slightly differing shades of dark blue (or dark green) above 150 mg/dL glucose. In contrast, a test device of this invention can produce dramatic color changes, blue to sea green to light yellow to red, over a range of 0 to 800 mg/dL glucose.

This invention provides compositions which allow the control of the production of a range of hues, especially for clinically important analytes.

III. Information Disclosure

U.S. Patent No. 4,490,465 discloses a test system for the determination of glucose having an extended range of measurement. The system contains at least one pyridine linked dehydrogenase and one non-pyridine linked dehydrogenase. However the indicators produced are said to be in equilibrium. Therefore, their reaction and subsequent color change could not be independently controlled.

DE 32 11 167 claims at least two enzymes systems each of which is independently capable of catalyzing the direct or indirect conversion of a substrate. The specification defines "independent of one another" to mean that, in the simultaneous presence of the systems that react with the substrate, the reaction through the second system takes place only after the coenzyme of the first system has been largely consumed.

DE 32 47 894 discloses a test system for the determination of NAD(P)H or substrates or enzymes reacting under the formation or consumption of NAD(P)H characterized in that it contains a single enzyme system which can react with several substances with different electrochemical potentials, in the presence of NAD(P)H. The color formation is controlled by the electrochemical potentials of the substances used and the system cannot be altered to produce different colors at difference analyte concentrations except by finding substances having different electrochemical potentials. Particular examples of electron acceptors include dichlorophenolindophenol and INT, (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride). The specification states that the test system can be impregnated into absorbent materials. A yellow component is added to the color seen by the incorporation of a background dye, titanium yellow.

Japanese Patent Application 59-106299 was published June 19, 1984. The application discloses a method for estimating NAD(P)H with oxidized glutathione in the presence of glutathione reductase and a color forming agent. Examples of color forming agents given are 5,5'-dithiobis(2-nitrobenzoic acid); N-(1-anilino-naphthyl-4)maleimide; Beta-hydroxyethyl-2,4-dinitrophenyldisulfide; 2,2-dithiopyridine; and benzimidazolyl maleimide.

European Patent Application 0-153-872 discloses a method for the determination of the reduced form of nicotinamide adenine dinucleotide phorphate which involves reacting NAD(P)H with (1) peroxidase or thiol oxide reductase and (2) diaphorase or an electron carrier in the presence of a chromogen and determining the pigment thus formed. These two reactions do not act on a common substrate.

None of these disclosures provide test compositions composed of two independent catalytic systems capable of generating changes in hue independently.

IV. Summary of the Invention

The invention provides a test composition for the visual determination of the concentration of an analyte in fluid sample, comprising:

(a) a common substrate generating system which in the presence of an analyte in a fluid sample will generate a common substrate selected from the group consisting of adenosine triphosphate, reduced flavin adenine dinucleotide, hydrogen peroxide, glycerol and glucose;

(b) a first independent catalytic system capable of generating a change in hue of a first indicator component by reaction with the common substrate; and

(c) a second independent catalytic system capable of generating a change in hue of a second indicator component by reaction with the common substrate;

whereby the generation of the change in hues of the first and second indicator components can be controlled to provide different hues at different analyte concentrations, the particular final hue produced by the test composition depending on the concentration of the analyte.

The composition can be dissolved to provide a test solution or incorporated in a carrier matrix to provide a test device format. The use of compartmentalization to prepare a test device is preferred.

V. Description of the Invention

Visual color matching is convenient and it can provide an acceptably accurate determination of analyte concentration without the need for expensive instrumentation. Color can be broken into components such as saturation, lightness and hue. Hue is commonly referred to as "color" e.g., whether something "looks" blue, red or yellow. Throughout the specification the term "hue" is used.

Generally one hue is associated with one form of a single indicator. Therefore in order to generate a range of hues, a number of different indicator molecules are required. The possible changes of hue are numerous. An indicator can change from one hue to another, one hue to colorless or from colorless to a hue. It is even possible that the indicator itself will not change but that the format of a device incorporating the test composition is such that there is an apparent change in hue of the device when contacted with a test sample containing the analyte. For example, the hue of the indicator could not be seen prior to the reaction of the test composition with the analyte but is visible after that reaction occurs. Because it is desirable that the change of final hue exhibited by the test device from one analyte concentration to another be as clear to the user as possible; the changes, colorless to a hue and a hue to colorless, have been preferred. When using two or more indicators, at least one indicator component is preferably changed from one hue to colorless. Otherwise the final hue of the test composition or device will move toward black as more hues are produced. While a composition containing two indicator components can be used, the use of three indicator components has been found to be advantageous.

It is particularly desirable to control the production and/or disappearance of indicators which in one form exhibit one of the primary hues: red, yellow, blue.

For example, consider a test composition containing indicators which exhibit the following change in hue in the order shown.

indicator 1) blue to colorless
indicator 2) colorless to yellow
indicator 3) colorless to red

If the indicators react in sequence, the apparent final hue of the test composition would be blue to colorless to orange (yellow + red).

However, if indicator 2 changed hue concurrently with indicator 1, the change in indicator 3 occuring later; the apparent final hue of test composition would be blue to green (blue + yellow) to yellow to orange (yellow + red) to red. A similar example is:

indicator 1) red to colorless
indicator 2) colorless to yellow
indicator 3) colorless to blue

If the indicators reacted in sequence the apparent final hue of the test composition would be red to colorless to yellow to green (blue + yellow). If the indicators reacted as described above, with two indicator changes being produced simultaneously the third change being delayed, the apparent hue of the test composition would be red to orange (red + yellow) to yellow to green (yellow + blue) to blue.

Both of these schemes containing simultaneous hue changes could produce a full spectrum RAINBOW. Such a RAINBOW would be desirable because it would provide the widest range of hues visible to the eye.

The problem is twofold: 1) to select the indicators having changes in hue induced independently to give the maximum change in hue, and; 2) to ensure that these indicators react in an orderly fashion depending only on the concentration of the analyte.

It has been found that a controlled range of hues can be produced by using two independent catalytic systems, each reactive with a common substrate to produce changes in hue of one or more indicator components. The systems are chosen such that the indicator(s) in one system are not in equilibrium with the indicator(s) in the other system during the time of the assay. Therefore, the final hue produced by the test composition when contacted by a particular analyte concentration can be controlled by the activities of the independent catalytic systems. The final hue produced can be controlled by the relative increase or decrease of concentrations of the components and catalyts in the test composition.

Each independent catalytic system is a system capable of generating one or more changes in hue or changes in apparent hue. Each system reacts with a common substrate. The common substrate is usually some pivotal substrate which participates in many catalytic reactions. For example, the common substrate can be reduced nicotinamide adenine dinucleotide, NADH; adenosine triphosphate, ATP; hydrogen peroxide, H_2O_2; glycerol; or any other moiety which can be produced from the analyte of interest and can participate in two independent reactions to produce changes in hue.

The terms catalyst and catalytic are used in their conventional sense herein. A "catalytic" reaction is a reaction in which the rate is changed by the addition of a "catalyst" but the catalyst itself is unchanged. The catalytic reactions referred to herein are usually enzymatic but can also include nonenzymatic reactions such as those catalyzed with phenazine methosulfate. The term "independent" means that the indicator components undergoing a change in hue in one catalytic system are not in equilibrium with the indicator component(s) undergoing change in the other catalytic system during the assay time involved.

The common substrate is generated from the analyte of interest by a common substrate generating system which is usually enzymatic. Table 1 shows analytes of clinical interest and useful enzymatic systems which can provide a common substrate for this test. These reactions, and the reaction components required, are well known and are presently the basis for many diagnostic reactions which generate changes in intensity of a single hue in response to analyte conentration.

TABLE 1

Analyte		Common substrate
glucose	glucose oxidase glucose dehydrogenase hexokinase/glucose-6-phosphate dehydrogenase	H_2O_2 or H_2FAD NADH NADH
cholesterol	cholesterol oxidase cholesterol dehydrogenase	H_2O_2 NADH
alcohol	alcohol oxidase alcohol dehydrogenase	H_2O_2 NADH
triglycerides α-amylase	lipoprotein lipase α-amylase	glycerol glucose

Each independent catalytic system is capable of generating one or more changes in hue by reaction with the common substrate. Useful pairs of catalysts for reaction with a common substrate are shown in Table 2.

Table 2

Common Substrate	Paired Catalysts
NADH	diaphorase/disulphide reductase
glycerol	glycerolkinase/glycerol dehyrogenase
glucose	hexokinase/glucose dehydrogenase

These systems are intended as examples only and are not to be interpreted as limiting in the invention. The components of these systems can be used to provide test compositions which operate according to the present invention. In addition, with color retardant can be added to the test composition to delay the reaction of the common substrate with the independent catalytic systems. Compounds such as potassium ferricyanide, 1-N-ethyl-4-methylquinoline iodide and analogs thereof can act as color retardants in a system designed to react with NADH.

The reduced form of nicotinamide adenine dinucleotide, NADH, has been found to be a particularly useful common substrate. This system is discussed in detail in EP 239 926.

Other components such as buffers, surfactants and polymers can be added to the composition. The pH is generally chosen to give good performance and stability to the reagents and is controlled by use of buffers. The use of buffers is preferred since the enzymes function better within the pH range of about 6.0 to 8. Choice of a buffer is within the skill of the art. Useful buffers include, but are not limited to, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES), 2-[tris(hydroxymethyl)methyl]amino ethanesulfonic acid (TES), 2-[N-morpholino]-ethanesulfonic acid (MES) and [3-(N-morpholino)propanesulfonic acid] (MOPS). Surfactants and polymers can be particularly useful in formulations containing a cationic tetrazolium salt as an oxidized indicator since surfactants such as polyoxyethylene ether, available under the trademark TRITON® X-100 from Sigma Chemical Co., and polymers such as polyvinylalcohol and polyvinylpyrrolidone, available as PVP K30 from Aldrich Chemical Co., appear to help solubilize the cationic indicator and prevent interaction with the other indicators in the test composition. Surfactants also improve wettability of the device in a dry phase formulation. Enzyme stabilizers such as bovine serum albumin, can also be added.

Test compositions of this invention can be used by dissolving the composition in a solution or they can be incorporated into a carrier matrix and affixed to a support member such as a polyester strip to provide dry reagent strips which are well known in diagnostics. These strips provide a format which is convenient to carry and store and which is particularly useful to home users such a diabetics. Preferred compositions of this invention generate a final hue, which can be associated with a particular analyte concentration, in less than about ten minutes.

The carrier matrix employed can be any of several known in the industry, as long as the matrix can be incorporated with the composition and it does not interfere with the reactions required for the production of color. These include paper and films such as those made from natural polymers, latexes, polyurethanes, silicones or combinations of these.

In order to obtain the clearest colors possible, a clear carrier matrix is preferred. Since common analytes for this invention are water soluble compounds such as those found in body fluids, carrier matrices which can contain water, such as hydrophilic carriers, are preferred. Suitable hydrophilic carriers include agarose, gelatin, poly(vinyl)alcohol, poly(propylimine), carrageenan and alginic acid. Other carriers could be used. Mixed multilayer carriers composed of an absorbent opaque matrix, such as paper, and a hydrophilic (e.g., gelatin) carrier layer can be advantageously used when the test components are compartmentalized.

In a preferred embodiment, a solution of 1.25% carbodiimide was used to crosslink a multilayer gelatin carrier matrix . This provides a format suitable for a whole blood glucose test which allows the blood sample to be removed from the test device by wiping the surface. Other coating materials, well known in the art, can be used to allow the device to be washed or wiped to remove a colored sample if necessary.

The hydrophilic carrier layer or layers are coated onto a rigid backing or support member such as polystyrene, polyester and the like. The backing can be opaque or transparent, although an opaque white backing is commonly preferred for visually read tests.

The number and types of components, which can be used in the independent catalytic systems described previously, is increased by the use of compartmentalization of possibly incompatible components in a test device format. Compartmentalization can take on many forms. Components can be separated by placing some in a separate layer, by solubilizing within one phase of an emulsion, by precipitation, by encapsulation and so forth. Some of the available methods are described in detail in the Examples.

In EP 239 926 a device for the determination of glucose is described which shows the different possibilities of compartimentalization.

The ultimate goal of the invention was to generate distinctly different hues at different analyte concentrations. It has been shown that this goal can be achieved with test compositions of this invention with a variety of methods as summarized below.

1) Choose oxidized indicators which will generate the desired hue or turn colorless upon reduction.

2) Choose indicators for reaction in a particular catalytic system which have differing reduction potentials which will control the sequence of reactions in that system.

3) Control the quantities of components in the independent catalytic systems so the components involved in one reaction sequence are essentially exhausted at chosen analyte concentrations.
4) Increase (or decrease) the amount of catalyst in a system. This will increase (or decrease) the rate of interaction or reduction of the indicator components by that system and therefore change the apparent hue at a particular analyte concentration.
5) Compartmentalize the components of the reaction system in a test device. The advantages of compartmentalization can include the ability to: change the apparent final hue of the device even when component concentrations are the same; utilize incompatible indicators or water insoluble indicators; and utilize enzyme systems normally inhibited by thiol indicators.
6) Add a color retardant which will delay the reactions of the independent catalytic systems.

The methods suggested above can be combined.

The invention will now be illustrated, but is not intended to be limited, by the following examples:

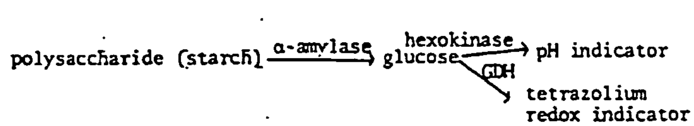
VI. Examples

Abbreviations

MPMS	1-methoxyphenazine methosulphate
DCIP	2,6-dichloroindophenol
ATP	adenosine triphosphate
NAD^+	Nicotinamide-adenine dinucleotide, lithium salt
NADH	reduced nicotinamide adenine dinucleotide
HEPES	buffer, N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid
BES	buffer, N,N-bis(2-hydroxyethyl)-2-aminoethane-sulfonic acid
Triton® X-100	surfactant, polyoxyethylene ether available from Sigma Chemical Co.
GDH	glucose dehydrogenase (EC 1.1.147) capable of producing NADH
U	International Units, a measure of enzyme activity (one U is the enzyme activity required to catalyze the conversion of one micromole of substrate per minute under specified conditions of temperature and pH
dL	deciliters
mL	milliliters
µL	microliters
g	grams
µ	microns

Example 1: Determination of Starch: glucose as a common substrate

A determination of starch can be made using the present invention to provide a visual endpoint of a different hue for different concentrations. Starch can be treated with amylase to generate glucose which is used as a common substrate for two independent catalytic reactions using hexokinase and glucose dehydrogenase (GDH) as enzyme catalysts. The overall reaction can be shown schematically as follows:



The specific independent reactions are:

$$\text{I.}\quad ATP^{2-} + \underset{\text{(glucose)}}{RCH_2OH} + H_2O \xrightarrow{\text{Hexokinase}} ADP^{2-} + \underset{\text{glucose-6-phosphate}}{RCH_2OPO_3^{2-}} + 2H^+$$

$$H^+ + \text{pH indicator} \longrightarrow \text{color change}$$

$$\text{II.}\quad \underset{\text{(glucose)}}{RCH_2OH} + NAD(P) \xrightarrow{\text{GDH}} \text{glucolactone} + NAD(P)H + H^+$$

$$NAD(P)H + \text{tetrazolium} \xrightarrow{\text{diaphorase}} NAD(P)^+ + \text{formazan}$$

A reaction mixture was prepared by combining 0.1 mM NAD, 0.025 mM MPMS, 0.5 mM p-nitroblue tetrazolium, 0.2% Triton® X-100, 10 mM ATP, 1 mM magnesium nitrate and 0.15 phenol red, and 10.1 mM TRIS buffer. The pH of the reagent mixture was adjusted to approximately 8.5 with ammonium hydroxide. The enzymes hexokinase (2.9 U/mL) and glucose dehydrogenase (9.9 U/mL) were added.

The pH indicator changes from red to faint yellow with decreasing pH; p-nitroblue tetrazolium changes from colorless to blue on reduction with NADH.

Aliquots of the solution were treated with glucose, allowed to react 5 minutes and the hue observed. The hues obtained with different levels of glucose (which can be related to the concentration of starch) were as follows:

glucose mg/dL	hue
0	mauve/red
72	pink
144	yellow
288	brown
432	darker brown
576	mauve

The red to yellow hues corresponded to the phenol red transition expected from the hexokinase reaction and the formation of blue (resulting in the brown to mauve) corresponded to the reduction of p-nitroblue tetrazolium by the glucose dehydrogenase pathway. The results show distinct hues which can be associated with the concentration of glucose, which can be related to the concentration of starch in a sample.

Example 2: Glycerol as a common substrate

Another common substrate, glycerol, can be produced in the assay of triglycerides.

$$\text{triglycerides} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol} + \text{free fatty acids}$$

The method is analogous to that of Example 1, where a kinase and a dehydrogenase were used. Glycerol kinase and glycerol dehydrogenase work at higher pH, so the buffer used was changed to BES. The redox indicator used was DCIP (blue to colorless) and the pH indicator was phenol red (red to yellow).

A solution having the following composition was prepared:

Reagent	NAD	$Mg(NO_3)_2$	ATP	Buffer	Triton X-100	phenol red	DCIP
final conc. (mM)	0.10	1.0	1.0	5	0.1%	0.15	0.15

The enzymes glycerol kinase (60 U/mL from E. coli, Sigma G 4509) and glycerol dehydrogenase (5.3 U/mL from cellulomonas, Sigma G 3512) were added.
The response of these solutions to glycerol at various times is shown below:

	Hue				
glycerol conc. (mM)	0	3	6	12	18
time (min.)					
1	mauve	blue	blue	blue	blue
5	mauve	blue	green	dark green	dark green
10	mauve	blue	blue/green	light green	light green
15	mauve	mauve/blue	blue	green	yellow/green
20	mauve	mauve/blue	blue/green	lime green	yellow

Claims

1. A test composition for the visual determination of the concentration of an analyte in a fluid sample, comprising:
   (a) a common substrate generating system which in the presence of an analyte in a fluid sample will generate a common substrate selected from the group consisting of adenosine triphosphate, reduced flavin adenine dinucleotide, hydrogen peroxide, glycerol and glucose;
   (b) a first independent catalytic system capable of generating a change in hue of a first indicator component by reaction with the common substrate; and

(c) a second independent catalytic system capable of generating a change in hue of a second indicator component by reaction with the common substrate;

whereby the generation of the change in hues of the first and second indicator components can be controlled to provide different hues at different analyte concentrations, the particular final hue produced by the test composition depending on the concentration of the analyte.

2. The test composition of claim 1 in which a third indicator component is also present and the first or second catalytic system is capable of generating a change in hue of the third indicator by reaction with the common substrate.

3. The test composition of any of claims 1 and 2 in which the change in hue of one of the first, second or third indicators is from a hue to colorless.

4. The test composition of any of claims 1 to 3 in which one of the first or second catalytic systems is capable of generating a yellow hue.

5. A test device for the visual determination of the concentration of an analyte in a fluid sample, comprising the test composition of claim 1.

6. The test device of claim 5 in which a third indicator component is also present and the first or second catalytic system is capable of generating a change in hue of the third indicator component by reaction with the common substrate.

7. The test device of any of claims 5 and 6 in which the change in hue of one of the first, second or third indicators is from a hue to colorless.

8. The test device of any of claims 5 to 7 in which one of the first or second catalytic systems is capable of generating a yellow hue.

Patentansprüche

1. Testzusammensetzung für die visuelle Bestimmung der Konzentration eines Analyten in einer flüssigen Probe, umfassend:

(a) ein gemeinsames Substrat erzeugendes System, das in Gegenwart eines in einer flüssigen Probe befindlichen Analyten ein gemeinsames Substrat erzeugt, das aus einer Gruppe, die aus Adenosintriphosphat, reduziertem Flavinadenindinucleotid, Wasserstoffperoxid, Glycerin und Glucose besteht, ausgewählt ist;

(b) ein erstes, unabhängiges katalytisches System, das befähigt ist, eine Veränderung im Farbton eines ersten Indikatorbestandteils durch Reaktion mit dem gemeinsamen Substrat zu erzeugen; und

(c) ein zweites, unabhängiges katalytisches System, das befähigt ist, eine Veränderung im Farbton eines zweiten Indikatorbestandteils durch Reaktion mit dem gemeinsamen Substrat zu erzeugen;

wobei das Erzeugen der Veränderung in den Farbtönen der ersten und zweiten Indikatorbestandteile kontrolliert werden kann, um verschiedene Farbtöne bei verschiedenen Analytkonzentrationen bereitzustellen, insbesondere Endfarbtöne, die von der Testzusammensetzung in Abhängigkeit von der Konzentration des Analyten gebildet werden.

2. Testzusammensetzung nach Anspruch 1, in der ein dritter Indikatorbestandteil auch vorhanden ist und das erste oder zweite katalytische System befähigt ist, eine Änderung des Farbtons in dem dritten Indikator durch Reaktion mit dem gemeinsamen Substrat zu erzeugen.

3. Testzusammensetzung nach irgendeinem der Ansprüche 1 und 2, bei der die Farbtonveränderung eines der ersten, zweiten oder dritten Indikatoren von einem Farbton zu farblos besteht.

4. Testzusammensetzung nach irgendeinem der Ansprüche 1 bis 3, bei der eines der ersten oder zweiten katalytischen Systeme befähigt ist, einen gelben Farbton zu erzeugen.

5. Testvorrichtung, zur visuellen Bestimmung der Konzentration eines Analyten in einer flüssigen Probe, umfassend die Testzusammensetzung nach Anspruch 1.

6. Testvorrichtung nach Anspruch 5, in der ein dritter Indikatorbestandteil auch vorhanden ist und das erste oder zweite katalytische System befähigt ist, eine Farbtonveränderung des dritten Indikatorbestandteils durch Reaktion mit dem gemeinsamen Substrat zu erzeugen.

7. Testvorrichtung nach irgendeinem der Ansprüche 5 und 6, in dem die Farbtonveränderung eines der ersten, zweiten oder dritten Indikatoren von einem Farbton zu farblos besteht.

8. Testvorrichtung nach irgendeinem der Ansprüche 5 bis 7, in dem eines der ersten oder zweiten katalytischen Systeme befähigt ist, einen gelben Farbton zu erzeugen.

Revendications

1. Composition d'essai pour la détermination visuelle de la concentration d'un analyte dans un échantillon de fluide, comprenant :
   (a) un système générant un substrat commun qui, en présence d'un analyte dans un échantillon de fluide, générera un substrat commun sélectionné parmi le groupe constitué par l'adénosine-triphosphate, le flavine-adénine-dinucléotide réduit, le peroxyde d'hydrogène, le glycérol et le glucose;
   (b) un premier système catalytique indépendant capable de générer un changement dans la teinte d'un premier composant d'indicateur par réaction avec le substrat commun; et
   (c) un second système catalytique indépendant capable de générer un changement dans la teinte d'un second composant d'indicateur par réaction avec le substrat commun;
   par laquelle la génération des changements de teintes des premier et second composants d'indicateur peut être contrôlée pour procurer différentes teintes à différentes concentrations d'analyte, la teinte finale particulière produite par la composition d'essai dépendant de la concentration de l'analyte.

2. Composition d'essai selon la revendication 1, dans laquelle un troisième composant d'indicateur est également présent et le premier ou le second système catalytique est capable de générer un changement dans la teinte du troisième indicateur par réaction avec le substrat commun.

3. Composition d'essai selon l'une quelconque des revendications 1 et 2, dans laquelle le changement de teinte d'un des premier, deuxième ou troisième indicateurs est un changement faisant passer d'une teinte à l'état incolore.

4. Composition d'essai selon l'une quelconque des revendications 1 à 3, dans laquelle l'un des premier ou second systèmes catalytiques est capable de générer une teinte jaune.

5. Dispositif d'essai pour la détermination visuelle de la concentration d'un analyte dans un échantillon de fluide comprenant la composition d'essai selon la revendication 1.

6. Dispositif d'essai selon la revendication 5, dans lequel un troisième composant d'indicateur est également présent et le premier ou le second système catalytique est capable de générer un changement dans la teinte du troisième indicateur par réaction avec le substrat commun.

7. Dispositif d'essai selon l'une quelconque des revendications 5 et 6, dans lequel le changement de teinte d'un des premier, deuxième ou troisième indicateurs est un changement faisant passer d'une teinte à l'état incolore.

8. Dispositif d'essai selon l'une quelconque des revendications 5 à 7, dans lequel l'un des premier ou second systèmes catalytiques est capable de générer une teinte jaune.